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Genetic variation in the Alpine chamois, with special reference to the subspecies *Rupicapra rupicapra cartusiana* Couturier, 1938

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Abstract

Genetic variation in 53 individuals representing 6 Alpine chamois *(Rupicapra rupicapra)* populations was investigated by starch gel electrophoresis, in order to determine the extent to which the endangered subspecies *R. r. cartusiana* differs from *R. r. rupicapra* and merits protection. Across all populations studied 10 of 55 loci screened were polymorphic and average heterozygosity was typical for a mammal but high for a large mammal. As measured by Nei genetic distances, the *cartusiana* population was the most distinct, but no loci diplaying fixed differences between populations were detected. It is concluded that the decision to protect the *cartusiana* population must depend on the cost of the protection.

Introduction

Chamois (genus *Rupicapra*) occur in mountainous regions of Central Europe and the Near East. In a recent taxonomic revision, two species were proposed, *R. rupicapra,* containing Alpine, Eastern European and Asian chamois and *R. pyrenaica* containing chamois native to the Cantabrain Mountains, Pyrenees and Apennines (Nascetti et al. 1985). Ten subspecies of chamois have been described (Couturier 1938; Lovari and Scala 1980), and this paper concentrates on the status and survival of one, *R. r. cartusiana.*

The subspecies *R. r. cartusiana* (hereafter referred to as *cartusiana*), named after the Chartreuse region of France, was described by Couturier (1938) who separated it from the geographically close *R. r. rupicapra* (hereafter referred to as *rupicapra*)on the basis of its horn shape, stockier build, darker winter coat colour and a series of skull traits (characteristics of the nasal and lacrymal bones, a long and narrow ethmoidal fissure and long premolar and molar tooth rows). At present, the population of the *cartusiana* subspecies is of unknown purity because of previous introductions (C. Berducou, pers. comm.) and numbers not more than 100 individuals (F. Roucher, pers. comm.). Furthermore, assuming that the *cartusiana* population is genetically distinct, its identity is under threat from two sources. First, a neighbouring, introduced, *rupicapra* population may expand further into the *cartusiana* area (see Fig. 1) and second, some local hunters hope to introduce more *rupicapra* within the current *cartusiana* range. In either case, the two subspecies might hybridize and the identity of the *cartusiana* subspecies might be lost for ever. The *cartusiana* population is rated as 'endangered' by I.U.C.N. (1986).

The aim of this study was to test the assumption, made in the previous paragraph, that the *cartusiana* population is genetically distinct from *rupicapra* populations and, if the populations proved different, to measure how different they are. This information could then be used in planning conservation measures for the *cartusiana* population.

As an objective method of measuring genetic variation, we used protein electrophoresis. In previous electrophoretic studies, several polymorphic loci have been identified in chamois (Nascetti et al. 1985; Miller and Hartl 1986; 1987).

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Material and methods

Samples of muscle (M), liver (L), kidney (K) and heart (H) (with some exceptions, see below) were collected by hunters in the course of normal hunting operations. The tissues were removed soon after the animals were dead and stored at −20 °C until processed for electrophoresis. Samples were collected from the *cartusiana* population itself, from four nearby *rupicapra* populations (see Fig. 1) and from one distant *rupicapra* population (Lombardia, Italy). Only muscle samples were obtained from the Lombardia population. Sample sizes are shown in Table 1.

Tissue samples were homogenized in buffer and centrifuged. The supernatants were loaded onto gels. Horizontal starch gel electrophoresis was carried out by conventional techniques, generally

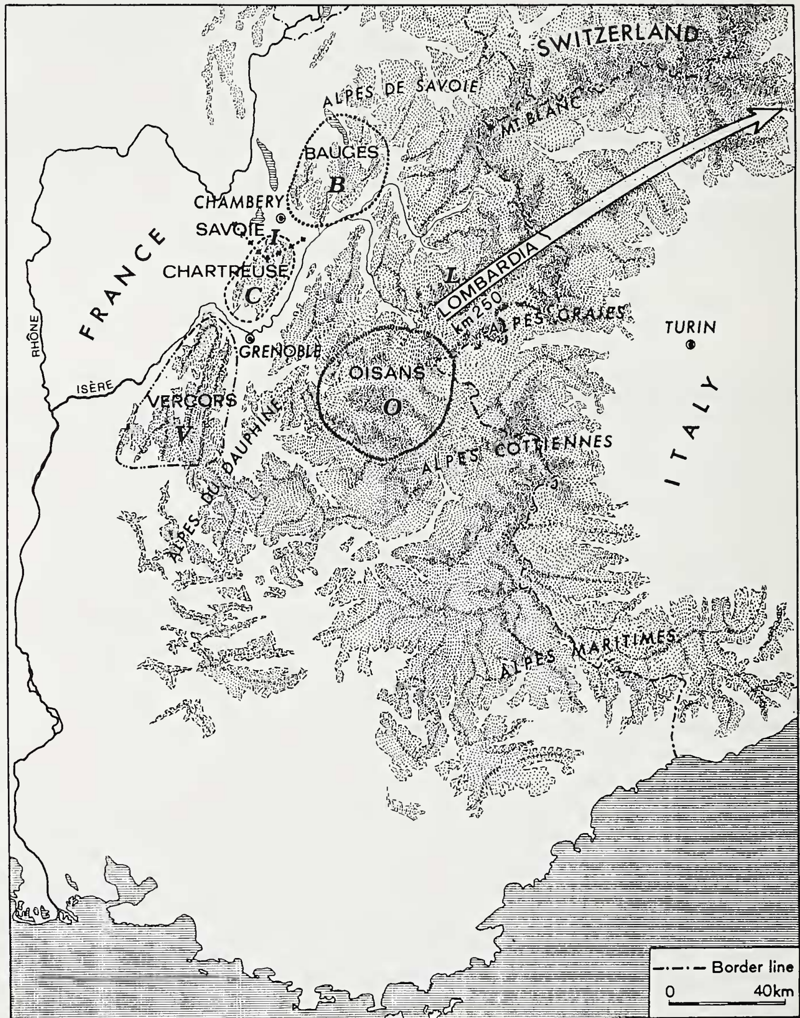

Fig. 1. Map of part of the French Alps, showing location of the *R. r. cartusiana* population (C) and of the neighbouring *R. r. rupicapra* populations which we sampled. Letters indicate sampling sites as coded in Table 1. The letter I indicates the population of introduced *R. r. rupicapra* which lives in the Chartreuse-Savoie region. This population was founded with 25 individuals from the Bauges (B) population and 4 individuals of Austrian ancestry (F. Roucher, pers. comm.). The Lombardia, Italy, sampling site lies approximately 250 km North East of the region shown

following buffer systems and staining recipes given by HARRIS and HOPKINSON (1976). The loci screened are listed below in the results section.

Relationships between the populations screened were studied by calculating genetic distances (NEI 1972) and conducting median hierarchical cluster analysis on the genetic distances using the SAS package (SAS INSTITUTE 1985).

Results

Where all four tissue types were available from an individual, we attempted to screen a total of 70 different loci. In the listings below, we have adopted the following conventions. Locus designations (−1, −2, −3) follow the notation of ALLENDORF and UTTER (1979) in which loci are numbered starting from the most cathodal seen. Additional descriptions are given in some cases. Peptidases are designated according to the substrate used (see HARRIS and HOPKINSON 1976). The symbols M, L, K and H after a locus indicate the tissue from which we preferred to score (or in the case of the first paragraph below, attempted to score) a particular locus. Because we had only muscle samples from the Lombardia population, many loci which we normally scored from other tissues were scored from muscle in the Lombardia population (indicated by a 'M' after the first choice tissue). For loci where no 'M' appears at all, we were unable to screen the Lombardia samples.

A total of 15 loci which we attempted to screen proved unsatisfactory because of insufficient enzyme activity or poor resolution of bands on the gel. These loci were acid phosphatase-3 (ACP-3, K), alcohol dehydrogenase (ADH, L), aldolase (ALD, M), diaphorase-1 and -2 (DIA-1, K, M and DIA-2, K, M), enolase (ENO, M), guanine deaminase (GDA, L, M), hexokinase-1, (HK-1, K, M), α-glycerophosphate dehydrogenase-2, (αGPD-2, H, M), inorganic pyrophosphatase (PP, M), peptidase-D and -E (PEP-D, K and PEP-E, K), phosphoglucomutase-3 (PGM-3, K), phosphoglycolate phosphatase (PGP, M) and xylose dehydrogenase, XLD, L).

No variation was found at 45 loci screened. These loci were acid phosphatase-1 and -2 (ACP-1, K and ACP-2, K, M), aconitase-2 (ACON-2, K), adenylate kinase-1 and -3 (AK-1, H, M and AK-3, H, M), creatine kinase-1 and -2 (CK-1, H, M and CK-2, H), liver esterase-1 and -2 (L-EST-1, L and L-EST-2, L), muscle esterase-2 (M-EST-2, M), ultraviolet esterase-1 and -2 (UV-EST-1, L and UV-EST-2, L) fructose diphosphatase-1 and -2 (FDP-1, M and FDP-2, L), fumarate hydratase (FH, L, M), glucose dehydrogenase (GDH, L, M), glucose-6-phosphate dehydrogen-

Table 1. **Allele frequencies at each polymorphic locus in each chamois population sampled, with average heterozygosities based on 55 and 43 loci**

Sample Site	Code	No. of animals	Polymorphic loci [commonest allele (s)]													
			ACON-1 (S)	(F)	ADA (S)	AK-2 (F)	M-EST-1 (S)	GOT-2 (S)	ME-1 (S)	NP (S)	PEP-B (S)	PGM-2 (M)	(F)	6PGD (F)	$\overline{H}$ (55)	$\overline{H}$ (43)
Chartreuse Savoie	I	11	0.60	0.40	0.91	1.00	1.00	0.96	0.91	1.00	0.82	0.50	0.50	0.77	0.042	0.039
Oisans	O	4	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.25	0.75	0.039	0.036
Bauges	B	11	0.95	0.05	0.96	1.00	1.00	0.82	0.90	1.00	0.68	0.59	0.18	0.73	0.037	0.034
Vercors	V	6	0.67	0.33	1.00	1.00	1.00	1.00	0.42	1.00	0.67	0.90	0.10	1.00	0.042	0.043
Lombardia	L	14	0.25	0.64	0.54	1.00	0.93	1.00	1.00	0.93	1.00	–	–	0.89	–	0.053
Chartreuse	C	7	0.93	0.07	0.71	0.93	0.93	1.00	0.71	1.00	0.64	0.64	0.36	0.21	0.049	0.050

ase (G6PD, K, M), glucose phosphate isomerase (GPI, K, M), glutamate oxaloacetate transaminase-1 (GOT-1, K, M), glutamate pyruvate transaminase (GPT, L, M), glutathione reductase (GR, K, M), glyceraldehyde phosphate dehydrogenase (GAPDH, H, M), α-glycerophosphate dehydrogenase-1 (α-GPD-1, H, M), hexokinase-2 and -3 (HK-2, K, M and HK-3, K, M), isocitrate dehydrogenase-1 and -2 (IDH-1, K, M and IDH-2, K, M), NAD-dependent isocitrate dehydrogenase (NAD-IDH, H, M), lactate dehydrogenase-1 and -2 (LDH-2, H, M and LDH-2, H, M), malate dehydrogenase-1 and -2 (MDH-1, K, M and MDH-2, K, M), malic enzyme-2 (ME-2, H, M), mannose phosphate isomerase (MPI, L, M), peptidase-A and -C (PEP-A, M and PEP-C, M), phosphoglucomutase-1 (PGM-1, K, M), phosphoglycerate kinase (PGK, K, M), phosphoglyceromutase-1 and -2 (PGAM-1, K, M and PGAM-2, K, M), pyruvate kinase-1 and -2 (PK-1, M and PK-2, L), sorbitol dehydrogenase (SDH, L, M) and superoxide dismutase-1 and -2 (SOD-1, H, M and SOD-2, H, M).

Polymorphism was found at 10 loci screened. These loci were aconitase-1 (ACON-1, K, M), adenosine deaminase (ADA, H, M), adenylate kinase-2 (AK-2, H, M), muscle esterase-1 (M-EST-1, M), glutamate oxaloacetate transaminase-2 (GOT-2, K, M), malic enzyme-1 (ME-1, H, M), nucleoside phophorylase (NP, L, M), peptidase-B (PEP-B, M), phosphoglucomutase-2 (PGM-2, L) and 6-phosphogluconate dehydrogenase (6PGD, K, M).

At most polymorphic loci we found two alleles. We called the most anodal F for fast and the less anodal S for slow. At PGM-2 and ACON-1 we found three alleles, so there was also a M for medium allele. All band patterns observed were consistent with the known quaternary structure of the enzymes involved. Observed allele frequencies and mean heterozygosity levels for each population are shown in Table 1.

From the electrophoretic data obtained we calculated Nei's genetic distances between each pair of populations screened, and we used median hierarchical cluster analysis to construct dendrograms showing the genetic relationships between populations. We performed these calculations twice, firstly including all 10 polymorphic and 45 monomorphic loci screened but on the French populations only, and secondly, in order to include the Italian population, we based the calculations on those loci which we were able to screen in muscle samples only. This reduced the sample of loci to 9 polymorphic loci plus 34 monomorphic loci. Genetic distances are shown in Table 2 while dendrograms illustrating genetic relationships between populations are shown in Fig. 2. In both analyses, the *cartusiana* population is genetically the most distinct of the populations screened.

Discussion

Among the French chamois studied, the population of the putative *cartusiana* subspecies (C) is genetically the most distinct (Table 2 and Fig. 2a). The Italian population screened (L) is of particular interest because, given its distant location from the French populations studied, one might expect it to be genetically distant from all of them as a result of local drift and selection processes. Contrary to this idea, when the Italian population is included in the analysis, it groups with the majority of the French populations, leaving the *cartusiana* population once again the most distinct (Table 2 and Fig. 2b).

How different is the *cartusiana* population from the *rupicapra* populations studied? If our electrophoretic study had shown that the *cartusiana* population grouped within the other French populations in the genetic distance dendrograms (Fig. 2), we would have demonstrated that there was essentially no difference between the *cartusiana* and *rupicapra* populations. If, on the other hand, we had found loci at which there were fixed differences between *cartusiana* and the other populations, we would have demonstrated a large difference between *cartusiana* and the *rupicapra* populations. In fact, we have found a

Table 2. **Genetic distances (Nei) between the chamois population studied**
Figures above the diagonal are based on 55 loci, while figures below the diagonal are based on the 43 loci which could be screened from muscle samples alone

Population	I	O	B	V	L	C
I		0.002	0.005	0.009	---	0.011
O	0.001		0.007	0.012	---	0.016
B	0.004	0.008		0.009	---	0.008
V	0.008	0.013	0.010		---	0.018
L	0.007	0.007	0.019	0.020		---
C	0.013	0.019	0.010	0.021	0.027	

situation between these two extremes. The *cartusiana* population is the most distinct studied, but the differences detected are all in allele frequencies. In a large sample of loci (55), we found none with fixed differences between *cartusiana* and *rupicapra*, and only one at which the *cartusiana* population has a different allele segregating (AK-2, and this was only in a single animal).

A second way to look at the data is to consider whether it supports the idea that the *cartusiana* population is a separate chamois subspecies. The subspecies classification is generally subjective and unsatisfactory and there are no objective rules for identifying a subspecies on the basis of genetic distances found from electrophoretic data. However, it is interesting to look at data from other species. Perhaps the most relevant study is the electrophoretic study of European red deer conducted by Gyllensten et al. (1983). In this study, genetic distances were compared between red deer of different named subspecies (which were originally separated on the basis of morphology). The survey involved 594

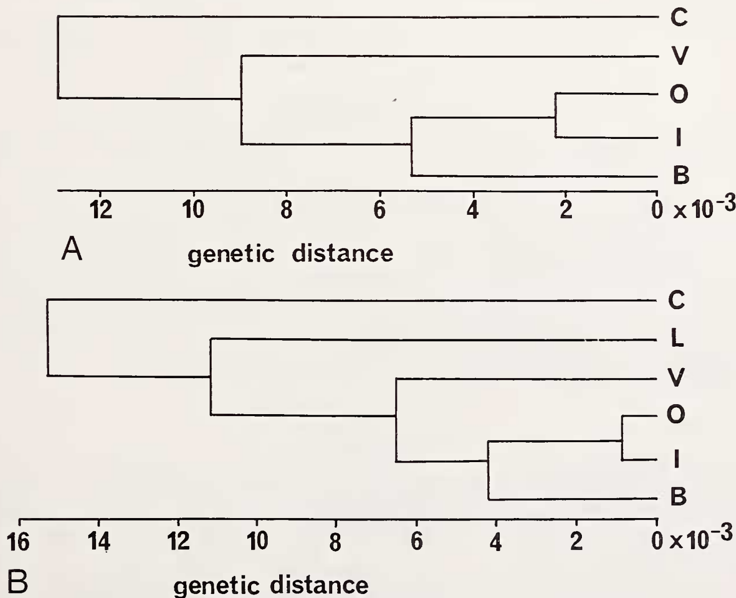

Fig. 2. Dendrograms, based on median hierarchical cluster analysis, showing the genetic relationships between the chamois populations studied. Site codes are as shown in Table 1. A: Shows the 5 French populations screened and is based on 55 loci, while B also includes the Italian Lombardia population studied and is based on the 43 loci which could be screened out of muscle samples

samples from 22 localities and 34 loci. Genetic distances between subspecies were variable but low compared with other investigated examples and the mean was 0.0164. The mean genetic distance between *rupicapra* and *cartusiana* populations in our study is 0.0133 (based on 55 loci but excluding Lombardia) or 0.0180 (based on 43 loci and including Lombardia). So the genetic distance between *rupicapra* and *cartusiana* is of the same order of magnitude as the distance between named red deer subspecies.

A third way to look at the data is to consider the amount of variation present in each population studied, by examining the average heterozygosity estimates given in Table 1. One might expect that the *cartusiana* population, being small and historically relatively isolated, would have lower levels of variation than other populations. However, among estimates based on either 55 or 43 loci, the *cartusiana* population has the highest estimated average heterozygosity among all the French populations studied. Only the Italian population, when included, has a higher estimated average heterozygosity.

To summarise the situation, we believe that the *cartusiana* population is genetically different from the *rupicapra* populations, and that for those who regard the subspecies status as important, the differences are large enough to justify calling the *cartusiana* population a separate subspecies. If the *cartusiana* population is protected, some of the existing genetic diversity of chamois will be maintained. Genetic diversity is probably important for the evolutionary survival of a species (FRANKEL and SOULÉ 1981). This benefit must be compared with the cost of protection. Protection would consist of, for example, stopping hunting of the *cartusiana* population for some time, preventing natural spread of *rupicapra* into the *cartusiana* population and not deliberately introducing a *rupicapra* population into the *cartusiana* range. We do not know what the costs of such protection would be, and so the cost-benefit comparison and the final decision is up to the French authorities. However, of the three protection measures mentioned, we feel that it would be very difficult to justify the deliberate introduction of a *rupicapra* population into the *cartusiana* range.

Our conclusions may be criticized because our sample sizes for each population are small. However, in general, estimates of genetic distances and average heterozygosities are more sensitive to the number of loci studied than to the number of individuals studied (NEI 1978; GORMAN and RENZI 1979) and we have studied a relatively large number of loci (55). We would like to have had samples from more individuals, especially of the *cartusiana* population. However, we would not like to encourage further hunting of an endangered population simply to obtain further samples. Small sample sizes are likely to be a common problem when laboratory techniques are applied to conservation problems, and the limitations of sample size should be considered before such studies begin.

There have been three previous studies of electrophoretic variation in chamois. NASCETTI et al. (1985) found 7 of 25 loci screened were polymorphic in samples from 5 populations (including 2 from the proposed *R. pyrenaica* species), and average heterozygosities ranging from 0.000–0.033. MILLER and HARTL (1986) found 8 of 41 loci were polymorphic in two Austrian populations, and average heterozygosities of 0.046 and 0.056. In a further survey of Austrian populations, MILLER and HARTL (1987) found 10 out of 42 loci were polymorphic, with average heterozygosities ranging from 0.035–0.047 (this data is also summarized in HARTL [1986]). In our study we found 10 out of 55 loci were polymorphic and average heterozygosities ranging from 0.034 to 0.053. Although the overall figures from the Austrian studies and our own are pleasingly similar, the Italian study differs strikingly and there are in fact many differences of detail among the various studies. We do not propose to discuss these in detail, but we suggest that three factors are responsible. First, the different groups have studied different populations of chamois, which may be expected to differ genetically. Second, the different groups have studied different combinations and numbers of loci, which are bound to influence estimates of the level of variation (GORMAN and RENZI 1979; HARTL 1985). Third, the different groups

may well have different levels of skill or standards. For example, although MILLER and HARTL (1986, 1987) found polymorphism at ACP-3 and PGM-3, we were unable to resolve these systems satisfactorily enough to score them. Nevertheless, we hope at some stage to be able to combine all the existing data sets to carry out a larger analysis of chamois populations.

Our results and those of MILLER and HARTL (1986, 1987) suggest that alpine chamois retain high levels of genetic variation compared with other large mammals. In a survey of electrophoresis studies of 184 species of mammal, NEVO et al. (1984) found that the mean average heterozygosity was 0.041 +/− 0.035 (S. D.). The average heterozygosity estimates given for Austrian and French chamois in the paragraph above suggest that alpine chamois have levels of variation close to the mean for all mammals. For reasons which are poorly understood, large mammals tend to have lower average heterozygosities than small mammals (WOOTEN and SMITH 1984; NEVO et al. 1984), so chamois are unusual among large mammals in having such high average heterozygosities.

Note added in proof

Under the auspices of the Office National de la Chasse, France, a similar selection of samples to ours has been screened electrophoretically by F. BONHOMME, Institut des Sciences de l'Evolution, Montpellier, France, and the results have recently become available. The survey was based on 25 loci and no evidence was found that the *cartusiana* population differed from neighbouring populations (C. BERDUCOU and F. BONHOMME, pers. comm.). We believe this difference from our study emphasizes the points we make in our discussion regarding the identity of loci studied, the differences in techniqes used in different laboratories and, especially, the need to study a large number of loci.

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Zusammenfassung

Die genetische Variabilität der Alpengemse mit besonderer Berücksichtigung der Unterart Rupicapra rupicapra cartusiana Couturier, 1938

Die genetische Variabilität von 53 Gemsen *(Rupicapra rupicapra)* aus sechs Populationen in den Alpen wurde mit Hilfe der Stärkegel-elektrophorese geschätzt. Geklärt werden sollte vor allem, wie weit sich die gefährdete Unterart *R. r. cartusiana* von *R. r. rupicapra* unterscheidet und Schutz verdient. 10 der insgesamt 55 untersuchten Genloci waren polymorph. Die durchschnittliche Heterozygotierate war für Säugetiere insgesamt typisch, für Großsäuger aber hoch. In den nach NEI berechneten genetischen Distanzen bilden die Tiere von *cartusiana* die am stärksten isolierte Population, wenn auch für keinen Genlocus ein völlig fixierter Unterschied gefunden wurde. Daraus wird gefolgert, daß der Schutz von *cartusiana* von den dadurch entstehenden Kosten abhängig gemacht werden sollte.

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